Differential Changes in the Mechanisms Controlling Luteinizing Hormone and Prolactin Secretion in Middle-Aged Female Rats

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Abstract

Serum luteinizing hormone (LH) and prolactin (PRL) concentrations were measured in young (3-4 month old) and middle-aged (10-12 month old) intact female rats on proestrus, in ovariectomized rats after two estrogen injections (estradiol benzoate; EB, 10 μ g/100 g body weight, s.c.) or after preoptic stimulation in EB-primed ovariectomized rats. Only animals showing regular 4-day estrous cycles were selected for the experiment. The magnitude of proestrous LH surge was significantly smaller in middle-aged than in young rats. Two BE injections, at noon on Days 0 and 3, in ovariectomized middle-aged rats failed to induce surges in LH secretion on Day 4 whereas the same treatment produced LH surges in ovariectomized young rats. The preoptic electrochemical stimulation (50 μ A for 60 sec) produced a prompt rise in serum LH levels in ovariectomized EB-primed young but not in middle aged rats. The preoptic stimulation with a larger current (200 μ A) induced LH secretion in middle-aged rats. In none of these situations serum PRL concentrations were different between young and middle-age rats. These results suggest differential aging rates in the preoptic mechanisms governing LH and PRL secretion in the rat. The function of the preoptic ovulatory center in responding to the estrogen positive feedback action and inducing LH secretion may become impaired and independent of the PRL control mechanism, even before the regular estrous cycle terminates.

As the female rat ages, regular estrous cycles are progressively replaced by irregular cycles, repeated pseudopregnancies, and acyclic constant estrous and anestrus states. Recent studies have shown that, prior to the senescent termination of the regular estrous cycles, the middle-aged rat already has changes in the hypothalmo-pituitary axis, which still allow cycling but ultimately

lead to infertility and eventual acyclicity. These include the finding that, on the day of proestrus in middle-aged regularly cycling rats, the critical period during which pentobarbital anesthesia could block the expected ovulation extended 1-2 hr beyond that of young cycling females (Van der Schoot, 1976). The proestrous surge of luteinizing hormone (LH) in the middle-aged rat appears to be altered as compared to that of the young in terms of the timing and/or the

peak value (Van der Schoot, 1976; Cooper *et al.*, 1980; Gray *et al.*, 1980; Wise, 1982; Nass *et al.*, 1984). Wise (1982) reported that the proestrous LH surge occurred significantly later with the peak concentration significantly lower in the middle-aged than the young rat.

The preovulatory increase in circulating estrogen is essential in inducing the proestrous surge in gonadotropins, as established by experiments using antisera to estradiol (Ferin *et al.*, 1969; Neill *et al.*, 1971). Increasing estrogen then triggers the activation of the medial preoptic area where the neural mechanism controlling the proestrous surge is believed to exist (Koves and Halasz, 1970). The aim of this study is to examine whether there is a change in sensitivity to the positive feedback effect of estrogen, and/or a change in the function of the preoptic area in controlling the secretion of LH in middle-aged rats, which, if any, may be responsible for reduced proestrous gonadotropin secretion. Since the aged non-cycling rat has a high serum prolactin (PRL) level (Huang *et al.*, 1976b) which may inhibit LH secretion (Smith, 1980), possible middle-aged changes in PRL secretion were also studied.

Materials and Methods

Animals

Multiparous middle-aged female rats (10–12 months old) of the Wistar strain were housed in a temperature-controlled room ($22 \pm 1^\circ\text{C}$) on a 14-h light, 10-h dark cycle (lights on 0500–1900 h). The animals were fed laboratory chows (MF, Oriental Yeast Co., Tokyo) and tap water ad libitum. Vaginal smears were examined daily and animals that showed at least 3 consecutive regular 4-day estrous cycles were selected for use in the following study. Regular 4-day estrous cycles were observed in 54% of the middle-aged animals ($n=48$). Nulliparous young rats (3–4 month old) showing regular 4-day estrous cycles were used as the control.

Experiment 1. Proestrous surges in LH and PRL.

The regular cyclers, 10 from the middle-aged group and 9 from the younger group, were implanted with intraatrial cannulae (#602–105, Dow-Corning Co., Midland, Mich.) through the jugular vein on the day of diestrous 2 under ether anesthesia, and placed in individual cages. On the next morning the proestrous vaginal smears (nucleated cells) were observed in all rats catheterized. Blood (approximately 250 μl) was collected through the indwelling cardiac cannula at hourly intervals from 1200 to 2000 h. The serum was separated after centrifugation and kept frozen at -20°C until assayed.

Experiment 2. Estrogen-induced LH and PRL surges.

To avoid the uncertain chronic influence of the possible different circulating titers of ovarian hormones on the LH and PRL release mechanisms, the regular cyclers in both middle-aged and young groups were ovariectomized and used 4 weeks later. The animal was injected subcutaneously with estradiol benzoate (EB; 200 $\mu\text{g}/\text{ml}$, dissolved in sesame oil) at a dose of 10 $\mu\text{g}/100\text{ g}$ body weight at noon (this day was referred to as Day 0). A second estradiol injection with the same dose was given at noon of Day 3, and the animal was implanted with an intraatrial cannula on the same day. Starting at noon of the following day (Day 4), blood was taken at hourly intervals till 2000 h. The serum was separated and kept frozen until assayed.

Experiment 3. Preoptic-induced LH secretion and PRL suppression.

Ovariectomized young and middle-aged rats were treated with EB on Day 0, as in experiment 2. On Day 3 the animal was anesthetized with sodium pentobarbital (35 mg/kg body weight) at 1330 h and placed in a stereotaxic frame. A coaxial stainless steel electrode (tip diameter 100 μm , insulated with epoxylite except the tip) was inserted into the medial preoptic area through a small hole made in the skull. The medial preoptic area was electrochemically stimulated by passing an anodal current of 50 or 200 μA for 60 sec between 1345–1400 h through the electrode. As the sham control, young ovariectomized EB-primed rats were anesthetized, placed in the stereotaxic frame and inserted with the electrode, but the current was not passed. Blood was collected through the indwelling cardiac cannula

at 1200 h and 1300 h and 0, 15, 30, 45, 60, 90, 120, 180 and 240 min after the stimulation. The serum was separated and stored frozen until assayed. After the last bleeding was performed the animal was perfused with 10 % buffered formalin and 3 % potassium ferricyanide and ferrocyanide solutions to make it possible to mark the site of the ferrous ion deposit. The site of stimulation was histologically verified in frozen sections of the brain.

Radioimmunoassay and statistical analysis.

Serum LH and PRL concentrations were measured by the double-antibody radioimmunoassay methods following instructions provided in the NIAMDD rat LH and PRL radioimmunoassay kits. The amounts of LH and PRL were expressed in terms of NIH-LH-S1 and PRL-RP-1, respectively. The intra- and inter-assay coefficients of variation were 4.11 and 8.60 % for LH and 4.38 and 9.24 % for PRL, respectively. The total amount of hormones released on proestrus or on Day 4 of EB treatments was estimated as the sum of hormone concentrations measured at hourly intervals and termed Σ LH or Σ PRL.

Two-way analysis of variance and Duncan's

multiple range test were used for statistical analysis.

Results

Experiment 1.

Figure 1 shows representative examples of serum LH profiles in individual middle-aged rats on proestrus. An LH rise was observed in each rat during the sampling period on the afternoon of proestrus, but the timing of the peak varied among rats. Similarly, the timing of PRL secretion on the proestrous afternoon varied among rats (data not shown). Thus, LH and PRL concentrations at a given time of the day varied too greatly to be treated statistically. Therefore, the mean LH and PRL concentrations of young and middle-aged groups (Fig. 2) at hourly intervals from the peak were compared.

The peak LH concentration ($p < 0.05$) and Σ LH ($p < 0.05$) on the afternoon of

Fig. 1. Serum LH profiles on proestrus in 6 individual rats. Note the individual variation in the timing of LH secretion.

proestrus were significantly lower in the middle-aged rat than in the young rat (Fig. 2a). In contrast, there was no apparent difference between the amount of PRL secretion on proestrus in young and middle-aged rats (Fig. 2b).

Experiment 2.

For the same reason as in Experiment 1, the hormone levels of young and middle-aged rats (Fig. 3) at a given interval from the highest value were compared. Twice EB injections on Days 0 and 3 induced a

significant rise in serum LH concentrations on the afternoon of Day 4 in young ovariectomized rats, but in middle-aged rats the serum LH rise was much smaller (Fig. 3a). However, the same treatment produced a serum PRL rise on Day 4 in both groups (Fig. 3b). There was no difference between the amount of PRL released in young and middle-aged rats.

Experiment 3.

Electrochemical stimulation of the medial preoptic area with a 50 μ A current produced

Fig. 2. Proestrous LH (a) and PRL (b) surges in young (●) and middle-aged (○) 4-day cycling rats. The values are expressed as the mean $\pm$ SEM (n=8-10). Smaller amounts of LH were released in middle-aged rats than in young rats while the amounts of PRL released were not different between two groups. *: $p < 0.05$ vs young.

a prompt rise in serum LH in EB-primed young ovariectomized rats (Fig. 4). The peak LH values were reached 30 min after the stimulation, then the level gradually declined over a course of 4-h sequential bleedings. Stimulation of the middle-aged rat with the same current (50 μ A) failed to induce a surge-like elevation in serum LH concentrations. Serum LH level rose in the middle-aged rat when a current of 200 μ A was given (Fig. 4a).

The preoptic stimulation (50 μ A) was effective in lowering serum PRL levels in both young and middle-aged rats, which were elevated due to anesthesia and surgical procedures in sham-stimulated young rats (Fig. 4b). Serum PRL levels or the amount of serum PRL reduction in young and

middle-aged rats were similar.

Discussion

In agreement with previous papers (Gray *et al.*, 1980; Wise, 1982; Nass *et al.*, 1984), the middle-aged female rat of our strain, showing regular 4-day estrous cycles, had a lower peak LH value during the proestrous surge than the young rat. A significant difference in the LH response to twice EB challenges was also observed between ovariectomized young and middle-aged rats, both of which had previously shown regular 4-day cycles. EB injections at noon on Days 0 and 3 produced a significant rise in serum LH in the young, but not middle-aged, rats

Fig. 3. Effects of EB treatments on Days 0 and 3 on serum LH (a) and PRL (b) concentrations on Day 4 in young (●) and middle-aged (○) ovariectomized rats. The values are expressed as the mean $\pm$ SEM ($n=6-10$). *, **: $p<0.05$, $p<0.01$ vs young, respectively. Note the absence of the LH surge in middle-aged rats. PRL surges were induced in both groups.

on Day 4. Wise (1984) recently reported the impaired positive feedback mechanism in ovariectomized middle-aged rats bearing subcutaneous implants containing estradiol. Our result, obtained by using a different method of estrogen administration, was in good accordance with her findings. These changes may participate in a reduced pro-

oestrous LH secretion in the middle-aged cycling rat.

In contrast to LH secretion, there was no age-related difference in the amplitude of proestrous PRL secretion or of EB-induced PRL secretion. These results may reflect a difference in the aging rate in the neural mechanisms controlling surge secretions of

Fig. 4. Effects of the medial preoptic stimulation on serum concentrations of LH (a) and PRL (b) in young and middle-aged ovariectomized, EB-primed rats. ▲ = sham-stimulated rats, ● = young rats (50 μ A), ○ = middle-aged rats (50 μ A), △ = middle-aged rats (200 μ A). The values are expressed as the mean $\pm$ SEM (n = 6–8). *: p < 0.05 vs sham. Note the absence of the LH surge response in middle-aged rats to 50 μ A stimulation, which effectively induced LH secretion in young rats. The same stimulation was effective in lowering serum PRL levels in both groups.

LH and PRL; the mechanism for PRL secretion may be more resistant to the influence of aging than that for LH secretion. Concerning the mechanism for reducing LH secretion in middle-aged rats, Wise (1982) suggested that an elevated PRL level may be involved, because a high PRL level is known to inhibit the LH surge (Smith, 1980). However, unchanged (the present study) or even lowered PRL secretion (Wilkes *et al.*, 1979) in middle-aged rats does not support this theory.

A question arises from the present results how the preovulatory surge in LH (though the peak is lower in the middle-aged than the young rat) and the 4-day estrous cycle are maintained in the middle-aged rat in which the positive feedback effect of estrogen is significantly decreased. A possible explanation is that the LH surge mechanism is exposed to a larger amount of estrogen in the middle-aged rat. In this case, a larger amount of PRL secretion would be expected to occur. However, this was not observed in the present study. Moreover, circulating estradiol concentrations are normal on proestrus in middle-aged rats (Smith *et al.*, 1982). Another possibility is that there may be some mechanism which compensates for the impaired estrogen feedback. Progesterone may be involved in the hypothesized compensatory mechanism, since progesterone has a positive feedback action on LH secretion in ovariectomized and EB-primed rats (Caligaris *et al.*, 1968), animals with brain lesions that block the estrogen positive feedback effect without interfering with the progesterone effect can maintain estrous cycles (Wiegand and Terasawa, 1982) and progesterone administration can reinstate estrous cycles in aged constant estrous rats (Huang *et al.*, 1976a).

Electrochemical stimulation of the medial preoptic area with a stimulus intensity of 50 μ A, which induced a surge in LH secretion in young rats, was again without effect in inducing LH secretion in middle-

aged rats. Wuttke and Meites (1973) reported a significant rise in serum LH levels after the preoptic electrochemical stimulation (100 μ A for 60 sec) in aged constant estrous rats and the inefficiency of the preoptic stimulation in aged pseudopregnant rats. However, they did not compare the effect of stimulation with young cycling rats. Our study clearly showed that, when the preoptic area was stimulated with a smaller current, such an inefficiency as observed in the pseudopregnant rat (Wuttke and Meites, 1973) could be seen even before the regular cycle ceases in the middle-aged rat. Wuttke and Meites (1973) also reported elevated serum PRL levels after the preoptic stimulation in aged constant estrous rats and these results seem to be contradictory to the suppression of PRL secretion which we observed. The reason for this discrepancy may be the differences between anesthetized and unanesthetized conditions, between cycling and constant estrous states, or in the stimulus parameters.

There are at least 4 possible age-related changes which could result in the lack of LH response to the preoptic stimulation in middle-aged rats. First, the excitability of preoptic neurons which are involved in LH surge control may be lowered in middle-aged rats. Effective stimulation with a larger current (200 μ A) supports this view. Second, changes in the neurotransmitter activity may be responsible. Kalra and McCann (1973) revealed that the blockade of the norepinephrine synapses prevents the release of LH induced by the preoptic stimulation in the rat. Thus, it is possible that lower norepinephrine activity in middle-aged rats (Wise, 1984) causes less effective synaptic transmission from the site of stimulation to the LHRH releasing apparatus. Third, changes in hypothalamic LHRH content and subneuronal distribution (Barnea *et al.*, 1980) may result in smaller amount of LHRH secretion induced by excitation of the LHRH releasing apparatus

in middle-aged rats. Finally, the change in pituitary responsiveness to LHRH (Smith *et al.*, 1982) may be involved. Thus, an amount of LHRH released from the hypothalamus would stimulate the release of a smaller amount of LH from less sensitive pituitaries in middle-aged rats. In any event, the aging change in the functioning of the preoptic ovulatory center in inducing LH secretion would ultimately lead to senescent estrous irregularity and anovulation.

The proestrous surges in LH and PRL are mediated through separate limbic and hypothalamic areas (Kimura and Kawakami, 1978), which may age at different rates. There are a number of studies showing differential age-related changes in localized brain areas or discrete nuclei in morphology (Hsu and Peng, 1978; Machado-Salas *et al.*, 1977), oxygen consumption (Peng *et al.*, 1977), neurotransmitter content and/or turnover (Meek *et al.*, 1977; Simpkins *et al.*, 1977; Wise, 1984) and in estradiol nuclear receptor concentration (Wise and Camp, 1984). Moreover, the preoptic stimulation (50 β A), which effectively induced LH release and PRL suppression in young rats, resulted only in inhibition of PRL secretion but not LH stimulation in middle-aged rats. This may also indicate differential aging rates in the neural mechanisms governing LH and PRL secretion, even if they reside within the same region or in a close vicinity.

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